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The Early Development of the Vaccinia–Rabies Recombinant Vaccine Raboral®

Richard Lathe and Marie Paule Kieny

Abstract

The recombinant vaccinia–rabies vaccine, now known as Raboral®, has been widely used in Europe and North America to control/eliminate rabies in the principal wildlife vectors, and thus prevent human transmission. The origins of this vaccine are sometimes forgotten, although the formulation has not changed substantially in almost four decades. This groundbreaking vaccine was assembled by a team at a very young (at that time) genetic engineering company, Transgène, in Strasbourg, France. The joint leaders of the rabies vaccine team reflect, 36 years later, on the trials and tribulations that went hand in hand with the construction of the vaccine.

Keywords: Alsace, glycoprotein, Pasteur, rabies, Raboral, vaccine, vaccinia virus, wildlife

1. Introduction

Rabies is a devastating disease that is inexorably fatal, unless – as Louis Pasteur demonstrated [1] – it is rapidly treated by immunization. Even so, the vaccination regimen that Pasteur recommended is punishing, and brought its own risks of hyperinflammation. In the mid-20th century rabies was widespread among wildlife (principally foxes in Europe and raccoons in North America), and even in developed countries the bite of an infected animal was a death warrant if not immediately and intensively treated.

With the advent of genetic engineering (GE) technologies in the 1980s, the renowned Institut Mérieux in Lyon, France – established in 1897 by Marcel Mérieux, one of Louis Pasteur’s assistants – brought a new task to the newly established GE company Transgène in Strasbourg: make a rabies vaccine. This chore was handed down to ourselves, two young postdoctoral scientists in the company.

Transgène, founded by Pierre Chambon and Philippe Kourilsky in 1979/1980, although being allocated half a floor in Pierre Chambon’s institute at the University of Strasbourg (**Figure 1**), was not quite ready: all available resources, at the very beginning, were allocated to setting up all the essential GE ingredients – oligonucleotide synthesis, DNA sequencing (done manually on thin polyacrylamide gels using radiophosphorus), basic sequence analysis, simple expression vectors in bacteria, yeast, and mammalian cells, even making our own restriction enzymes. Other GE



Figure 1.

Transgène and the Wistar Institute. (Left) The Laboratoire de Génétique Moléculaire des Eukaryotes (LGME) under Pierre Chambon at the Faculty of Medicine, University of Strasbourg (founded 1538), France, where Transgène was housed (6th floor) during the development of the rabies vaccine (1980s). The building was constructed in 1964 and was demolished in 2011 {[https://www.archi-wiki.org/Adresse:Facult%C3%A9_de_m%C3%A9decine_\(Strasbourg\)](https://www.archi-wiki.org/Adresse:Facult%C3%A9_de_m%C3%A9decine_(Strasbourg))}. (Right) Wistar Institute of Anatomy, Philadelphia, USA. The Wistar was founded in 1892, and the Wistar Institute Building was opened in 1894. Image courtesy Jeffrey M. Vinocur 2006. Images are reproduced under Wikipedia Creative Commons Attribution-ShareAlike License.

companies were well ahead, and had cloned and expressed key molecules such as growth hormone, interferons, and hepatitis B surface antigen. We were both over-optimistic and underweight.

2. Construction of the Vaccine

But we set to. The Scientific Director of Transgène, Jean-Pierre Lecocq, established a collaboration with researchers at the Wistar Institute (**Figure 1**) under Hilary Koprowski (Philadelphia) who had obtained, for the first time, a cDNA copy of rabies virus glycoprotein at the end of 1981 [2, 3] – the key antigenic determinant of this virus. Beginning in early 1982, we expressed the cDNA in *E. coli*, *B. subtilis*, yeast, mammalian cells (reviewed in [4]). The pressure was on, we worked 7 days a week, so did our key technical staff; we were fuelled by Martha Argerich interpreting J.S. Bach on the tape player until long after the sun set over the Vosges hills (**Box 1**). The extracts were sent to the rabies expert in Philadelphia, Tadeusz Wiktor. He systematically vaccinated lab

It is singularly appropriate that the recombinant rabies vaccine should have been developed in Strasbourg, the central town of the province of Alsace, France. Louis Pasteur, famed for the first rabies vaccine, was born in the Jura hills (the southern extension of the Vosges – the hills of Alsace) and was Professor of Chemistry at Strasbourg University (formerly known as the Université Louis Pasteur) from 1849 to 1854, where he married Marie (also known as Louise) Laurent (the daughter of the Rector of the University), who for many years acted as his scientific assistant. The first patient to be treated against rabies, Joseph Meister (9 years old), traveled from Alsace to Pasteur's laboratory (then in Paris) for treatment [1]. The first fox to be inoculated with the new recombinant vaccine was in Malzéville, over the Vosges hills just 100 km from Strasbourg.

Box 1.

Rabies, Pasteur, and Alsace.

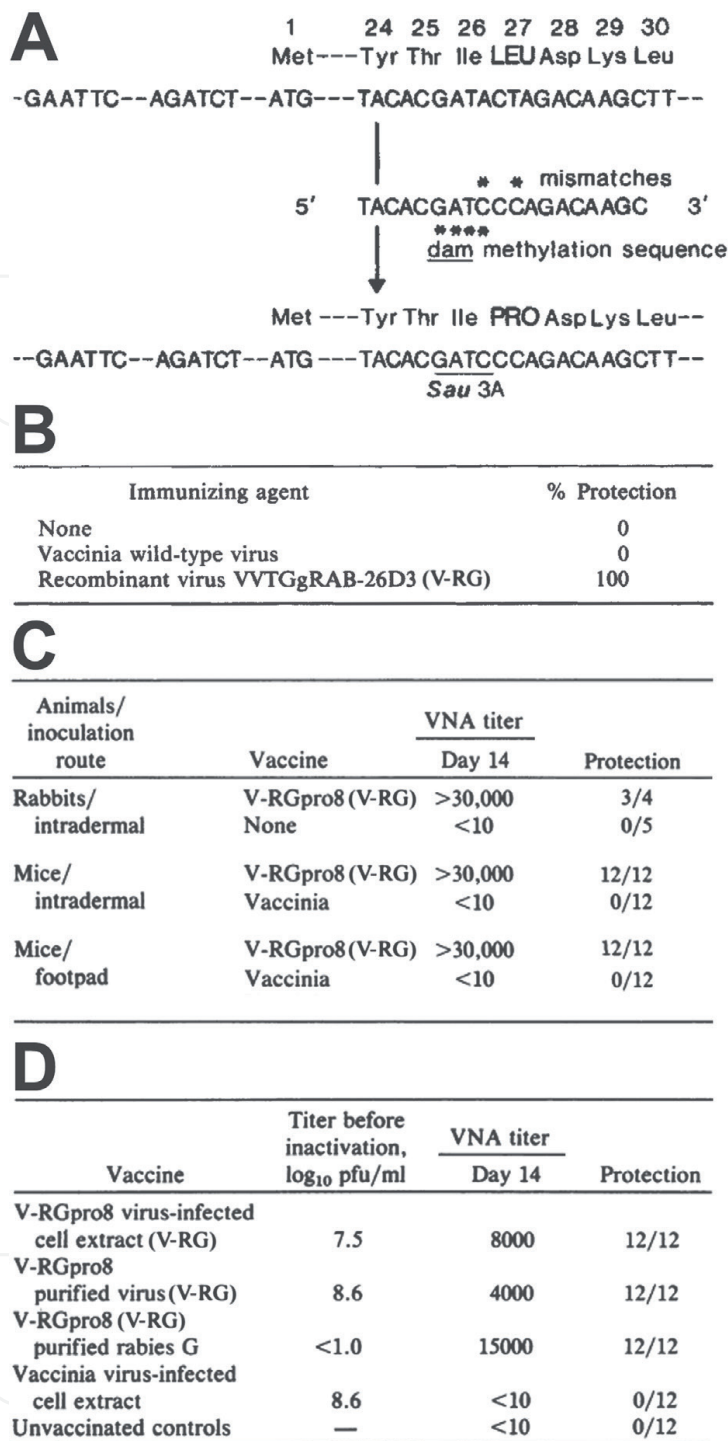


Figure 2.
Construction and Activity of the Vaccinia-Based Recombinant Rabies Virus Raboral® VR-G. The recombinant was variously known as VR-G, VVTGgRAB-26D3, and VR-Gpro8. (A) Correction of a mutation in the N-terminus of the rabies glycoprotein coding sequence. Panel adapted, with permission, from [6]. (B) Protection from rabies using the live recombinant vaccine [6]. Mice were inoculated (intradermal) with 5×10^5 PFU of VR-G or wild-type vaccinia, and challenged on day 15 by intracerebral inoculation of CDC culture-adapted street rabies virus (2400 LD₅₀ units). All vaccinated animals survived. Panel adapted, with permission, from [6]. (C). Protection of rabbits and mice: inoculation was with 2×10^8 PFU (intradermal) or 5×10^7 (footpad) of VR-G; intracerebral challenge at day 14 was with 2400 (mice) or 24 000 (rabbits) mouse LD₅₀ units of MD5951 street rabies virus. Panel adapted, with permission, from [7]. (D) Protection using inactivated VR-G. Vaccines were inactivated with β -propionolactone before intraperitoneal administration into mice. Challenge at day 14 was with 240 LD₅₀ units of MD5951. Panel adapted, with permission, from [7].

animals (mostly mice and hamsters) with the extracts, and then challenged them with street rabies virus. None survived.

We were dismayed, and ready to give up. Two developments changed everything. First, at a chance meeting with Peter Curtis (Wistar) he advised that there was a possible problem with his cDNA sequence – there appeared to be a mutation. Second, we were impressed by the growing achievements of recombinant vaccinia virus (the basis of the smallpox vaccine), inspired by Enzo Paoletti in 1983 [5], in eliciting immunity beyond what could be obtained with bacteria, yeasts, or even mammalian cells.

The mutation in the rabies glycoprotein cDNA was indeed suspect – a Pro to Leu mutation near the beginning of the mature protein sequence at a position that (to our minds) seemed to resemble known mutations at the beginning of the oncoprotein RAS (Gly to Asp, or Gly to Val) that entirely transform the structure and activity of the protein. The first thing we did was to correct the mutation. Something we had never done before, and this took months. In the key *Nature* paper the reader will see that, because our techniques were challenged, we took advantage of an artificial site for Dam methylase (that governs mismatch repair in *E. coli*) site to tip the balance in our favor (unheard of today) (**Figure 2A**). But it worked! We wonder how many rabies glycoprotein sequences circulating in the GE world today bear that same signature alteration.

The second thing we did was to get vaccinia up and running as a vehicle. We got into collaboration with Robert Drillien and Danièle Spehner at the Institut de Virologie on the same campus, and they supplied us with a microgram of vaccinia virus DNA. The key thymidine kinase gene – required for recombinational exchange with the vaccinia genome – had already been cloned by Robert, but we lacked a promoter to drive expression. We wanted the 7.5 K gene promoter, that had been worked up by researchers in the USA, but starting with only 1 microgram of DNA (the vaccinia genome is ~190 kb in length), given the state of the technology, cloning was near to impossible. In the event we turned to calculations of insert size, vector size, and absolute concentrations based on the physicochemistry of ligation [8, 9], and brewed up an optimum ligase mix. Only four clones were obtained. Two were 7.5 K in one orientation, and two were the same promoter in the other orientation! Were we lucky? No, in retrospect we would have got nothing had we not used the mathematics of the ligation reaction to get what we needed.

From there it moved quite rapidly. Ligate the promoter to the modified rabies G coding sequence, insert the construct into the vaccinia TK gene on a plasmid – then transfect the recombinant (TK-negative) plasmid into vaccinia virus-infected cells, and select chemically using bromodeoxyuridine (that kills TK-positive viruses). And we had our first vaccinia–rabies recombinant ('26D3').

This sped off to Tad Wiktor in Philadelphia. We waited what seems like an eternity, but he reported quickly back with his preliminary results: 'this is the best rabies vaccine I have ever seen' – it protected mice against severe rabies challenge (**Figure 2B–D**) better than any other rabies vaccine. And so it turned out – first published in September 1984 ([6, 7]; reviewed in [10–12]).

What lessons can be drawn, if any? Our first thought is that we worked as a team, there was no academic infighting, no bickering about authorships that is too common today, and we all worked day and night to see the project through. Even technical staff were there late in the evening and at weekends. Second, collaboration is essential: we could not have done this project without the participation of scientists both near (Robert Drillien and Danièle Spehner) and far {Tadeusz Wiktor (**Figure 3A**), Peter Curtis, and Hilary Koprowski}. Third, we wonder whether Martha Argerich and J.S.

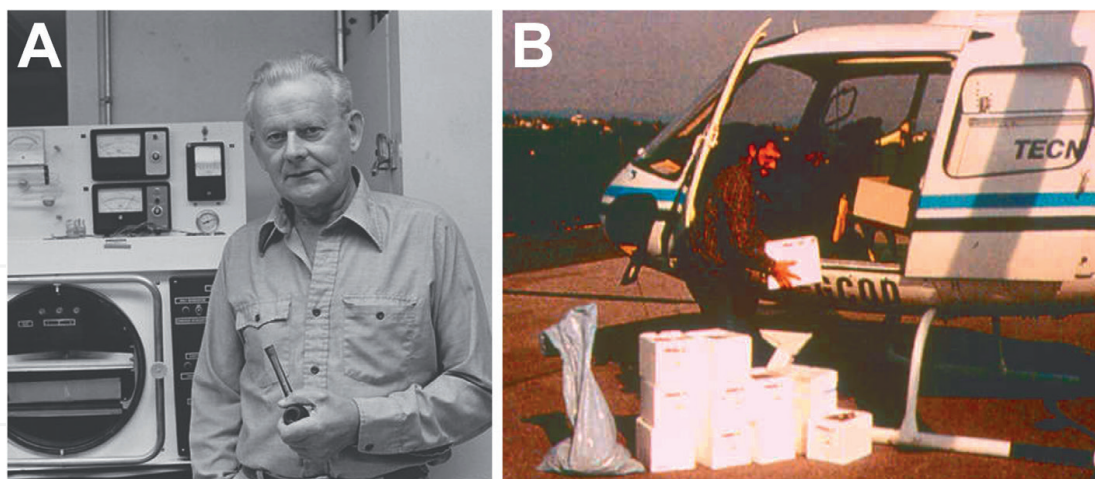


Figure 3.
Pioneering Rabies Vaccination. (A) Tadeusz J. Wiktor (1920–1985), rabies expert, Wistar Institute, who first tested the recombinant for efficacy. Photo courtesy of the Wistar Institute. (B) Loading vaccine baits into a helicopter during the first wildlife vaccination campaigns (Belgium, ca 1988; image collection M.P.K.).

Bach played a role. In this day of *P* values, we would need to run the entire project again with a different musical background.

3. Vaccination of Wildlife

The vaccine was originally envisaged for human use, and steps were taken in this direction in collaboration with the Institut Mérieux. However, there was reluctance to introduce a new vaccinia-based vaccine for human use because smallpox, the original target of vaccinia, had recently been declared eradicated by the World Health Organization (WHO) [13]. A committee of international experts including WHO representatives, who met in Bethesda, MD, in November 1984, did not envisage early human use [14]. However, because rabies is transmitted to humans principally from domestic dogs – that themselves acquire the infection from foxes (in Europe), and from raccoons and coyotes (in North America) – the next step was to try the new vaccine out for efficacy in animals. With support from Philippe Desmetre at the Institut Mérieux, Transgène was in touch with Pierre-Paul Pastoret in Belgium and colleagues at the French Ministry of Agriculture Rabies Research Station in Malzéville close to Nancy, and this was soon followed by the demonstration that oral administration of the vaccine (10^8 pfu) to foxes (**Figure 4**) gave complete protection against lethal challenge, and almost complete protection following administration in home-made bait [15], quickly followed by oral protection of raccoons in the USA by colleagues at the Wistar Institute [16].

This was expanded to small-trials followed by large-scale campaigns to eradicate sylvatic rabies in Europe and North America by dropping baits (e.g., chicken heads or artificial baits) seeded with live recombinant virus from helicopters (**Figure 3B**) according to a carefully planned routine (taking in mind the number of wildlife species per square kilometer, and the transmission factor *R* – what percentage of animals do we need to vaccinate to block propagation? – of current interest given the COVID pandemic). It worked. The first trials (**Figure 5**) demonstrated wide uptake by foxes and downturn of rabies cases. Using this vaccine, rabies has now been widely eliminated in many European countries; substantial reductions in rates of rabies in wildlife



Figure 4.
Experimental vaccination of foxes, Malzéville, France, ca 1986 (image collection M.P.K.).

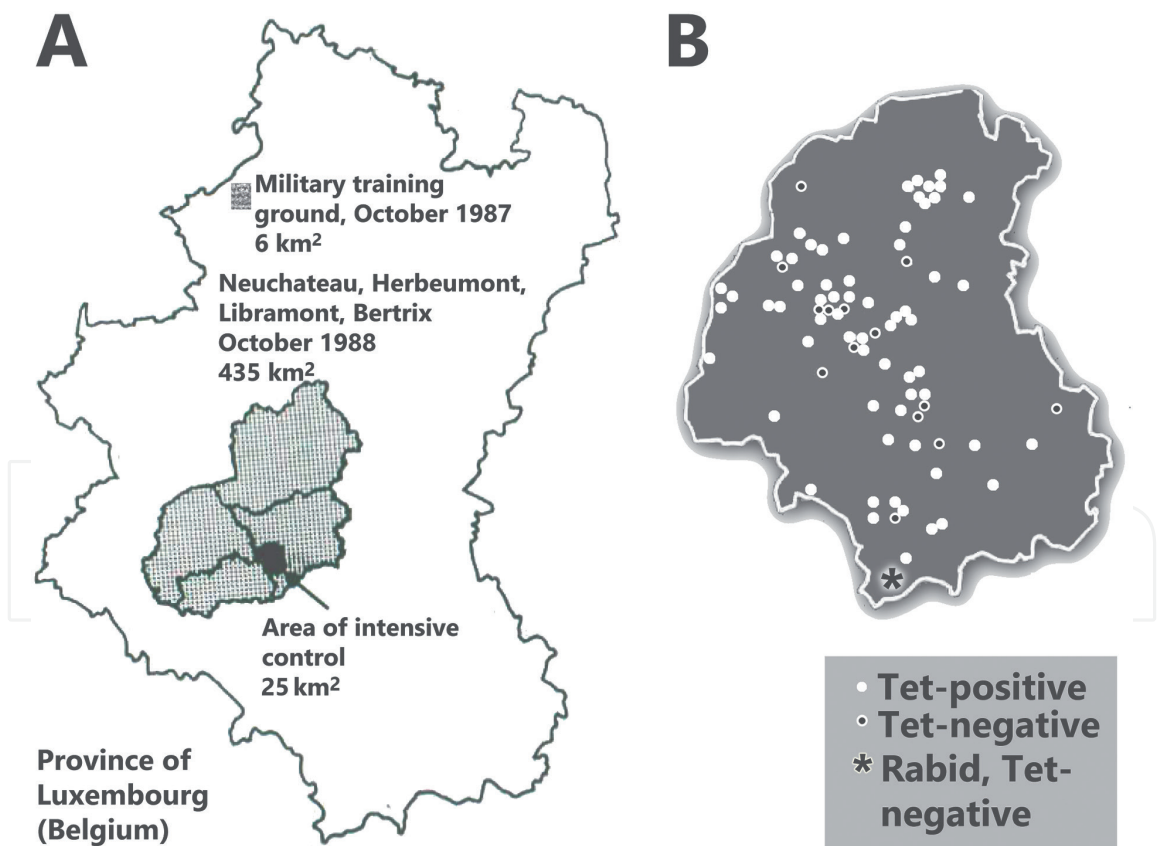


Figure 5.
First wildlife vaccination campaigns. Baits containing VRG were prepared from fish oil and protein. (A) Test areas in Belgium, 1987–1988. Vaccines were distributed at 15–50 baits per km²; uptake ranged from 27% at day 4 to 94% at day 223 (image collection M.P.K.). (B) Larger-scale field trial in a 2200 km² area of Belgium; distribution was at 15 baits per km²; the area was vaccinated three times (1990–1991). Baits contained a tetracycline biomarker, and uptake determined from the presence of tetracycline (Tet; UV fluorescence in jaw) was 81% after the third phase. The single rabid fox detected after the campaign, at the periphery of the baited area, was Tet-negative. Image adapted, with permission, from [17].

have been achieved in several areas of Eastern USA and Canada (**Table 1**) where active vaccination campaigns are ongoing (<http://www.raboral.com/about-rabies/raboral-v-rg>). Over 250 million doses of Raboral® have been distributed worldwide [19]. Although highly successful in Europe [18], campaigns in the USA and Canada are constrained because of rabies re-emergence through long-distance movements of carrier species such as arctic foxes [19].

Europe (including Russia)	
Austria	Eliminated (2006)
Belgium	Eliminated (1999)
Bulgaria	Reduction in seeded areas
Czech Republic	Eliminated (2002)
Estonia	Eliminated (2009)
France	Eliminated (1998)
Germany	Eliminated (2006)
Hungary	Reduction in seeded areas
Italy	Eliminated (1986/1995); then reduction
Latvia	Reduction in seeded areas
Lithuania	Reduction in seeded areas
Luxembourg	Eliminated (1999)
Poland	Reduction in seeded areas
Kaliningrad (Russia)	Reduction in seeded areas
Russia	Experimental
Slovakia	Eliminated (2006)
Slovenia	Reduction
Switzerland	Eliminated (1996)
Ukraine	Reduction
North America	
Canada	Reduction in seeded areas
United States	Reduction in seeded areas
Middle and Far East	
Israel	Reduction in seeded areas
Tunisia	Experimental
India	Experimental
China	Experimental

**In many cases Raboral® was used in conjunction with conventional attenuated viruses, although Raboral® is the only vaccine licensed for use in the USA (<http://www.raboral.com/about-rabies/raboral-v-rg>). Data: key reviews are by Freuling et al. [18] and Maki et al. [19].*

Table 1.
Wildlife Vaccination Campaigns Using Raboral®.*

4. Vaccinia-based vaccines for human use

The vaccinia–rabies recombinant has never been approved for human use, even though vaccinia virus has been used widely across the world in our populations to eradicate smallpox, and the vaccinia–rabies recombinant is very much attenuated compared to standard strains of vaccinia. Notwithstanding, intense efforts have been made to develop vaccinia as an anticancer agent for direct prophylactic or curative use in human [20–23], but for recombinants expressing viral antigens few clinical trials have been carried out (with some exceptions; e.g., [24]). Efforts in the 1990s were invested into the development of viral vectors based on vaccinia derivatives such as modified virus Ankara (MVA) [25], or fowlpox or canarypox [26]. More recently, high levels of protective antibodies have been obtained against SARS coronavirus using vaccinia recombinants in experimental animals (e.g., [27–29]), and, given the threat of COVID-19, MVA-based recombinants are being actively explored in California [30] and Germany (<https://www.vfa.de/de/englische-inhalte/vaccines-to-protect-against-covid-19>) as potential vaccines against SARS-COV-2, the etiologic agent of COVID-19.

One potential way to circumvent safety concerns may be to employ inactivated recombinant vaccinia virions that display viral antigens at their surface: very substantial levels of protection were observed with chemically inactivated recombinant vaccinia–rabies virus [7], and this might afford an entry route for new human vaccines based on vaccinia – perhaps even at some stage including Raboral® or further attenuated derivatives, given that rabies in human remains a major concern in several regions such as India [31].

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